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Clinical significance of blood phosphorus compounds

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THE CLINICAL SIGNIFICANCE OF BLOOD PHOSPHORUS COMPOUNDS

"A T H E S I S"

Presented to the Faculty of the College of Medicine
of the University of Nebraska in Partial Fulfillment
of the Requirements for the Degree of Doctor of Medicine.

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April 12, 1934

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THE CLINICAL SIGNIFICANCE OF BLOOD PHOSPHORUS COMPOUNDS

Introduction

The purpose of this article is to review the known phosphorus compounds of the blood and to present their clinical significance in a way understandable to the practitioner. It has been my privilege to spend a year in the research laboratory studying the blood phosphorus compounds. Much of the known chemistry is as yet of theoretical value only and much is yet to be learned, however, rapid advances are going on at the present time in this branch of research. When the final chapter is written on blood chemistry of the phosphorus compounds the cause and perhaps cure of diabetes mellitus, rickets, the primary muscular atrophies, many disorders of the acid-base equilibrium and many other less important pathological conditions, will be solved. However much important knowledge is already known about the variations of the blood phosphorus compounds in disease, which is too frequently entirely overlooked or disregarded by the practitioner in his treatment of such diseases. This paper will endeavor to point out the specific diseases where a study of the blood phosphorus compounds is helpful in the diagnosis and treatment; a discussion of phosphorus requirements, absorption and excretion is also included.

In the chapter entitled "Known Phosphorus Compounds of the Blood", a brief discussion of the different known compounds is given and also briefly how they are studied and the Kuttner and Lichtenstein modification of the Kuttner and Cohen colorimetric determination of inorganic blood phosphorus is given.

Known Phosphorus Compounds of the Blood

The phosphorus compounds of the blood may be divided into three main groups, namely, phospho-proteins, phospholipids and acid soluble phosphates.

Phospho-proteins

Little is known of this group of phosphorus compounds because of their great complexity and the difficulty of isolation because of their extreme instability. However, it is believed that a protein phosphoric acid or protein phospholipin complex exists in the blood plasma contributing to the vital phenomena as well as in the cytoplasm of all cells.

The blood platelets and plasma fibrinogen contain a phospho-protein compound, so clotting of the blood is dependant on phospho-proteins.

Another group of phospho-protein compounds are the nucleoproteins in which phosphoric acid is a component of the nucleic acid group. In agranulocytosis, Jackson, Parker, Rinehart, and Taylor, (24) have obtained good results in some cases by administration of adenine sulphate, guanine hydrochloride or pentose nucleotides (6.7 gram in 100c.c. saline solution intravenously and intramuscularly for four days or until improvement begins.)

Phospholipids

This fraction is obtained by alcohol-ether extraction. Normally about two-thirds are lecithin. The rest is cephalin.

Bloor (6) points out that the phospholipid fraction of the red blood cells is fairly constant under different conditions, as is also true of the phospholipid fraction of all tissues, but not true of the plasma. In experiments on dogs

and rabbits, he shows how high fat diets produce high phospholipid and cholesterol levels in the blood, while the neutral fat remains at a fairly constant level. He suggests that the phospholipids and cholesterol play a double part as an aid in the transport of fat and as fat metabolites.

Meigs, Blatherwick and Cary (43) demonstrated that there were probably no phosphorus compounds in the blood plasma other than the inorganic phosphate and phospholipids. They also showed that milk fat and milk phosphorus are derived from phosphotides. They contended that fat absorbed from the alimentary tract is very soon largely or completely converted into phosphatide by the red blood corpuscles. This they attributed to the inability of the tissues to receive their supply of fat from the blood except in the form of phosphatide. The body can synthesize phosphatide from tri-glycerides and inorganic phosphate. They also contended that phosphorus from the digestive tract reaches the general circulation only in the form of inorganic phosphate, all organic phosphorus compounds being synthesized within the body cells.

From the above it is seen that the phospholipids are very important in fat metabolism. In diseases characterized by deranged fat metabolism, phospholipid studies should reveal considerable. The phospholipid changes in diabetes will be discussed in the chapter on diabetes. In arterial sclerosis, fatty degenerations, obesity and similar conditions where the fat metabolism is abnormal, we can expect to learn much by further studies on the phospholipid fraction of the blood.

Bloor (5) demonstrated an increased lipid phosphorus

of the plasma in acute hemorrhage. He also showed that the lipid phosphorus fraction is greater in newly formed cells than in old ones. The importance of lecithin in the permeability and structure of the lipid membrane of the red corpuscles has long been known.

Acid Soluble Fraction of Blood Phosphates

This fraction is obtained by precepitation of the blood proteins by any of the protein precipitants. Trichloroacetic acid is commonly used. The filtrate contains inorganic phosphorus, pyrophosphate and the acid soluble phosphorus esters.

Orthophosphoric acid was isolated in the form of its acid potassium salt from muscles by Valenciennes and Fremy (53) 74 years ago. It has several important functions. Its role in bone and sugar metabolism will be discussed in the chapters on rickets and diabetes, respectively. Its importance in the acid base balance is discussed in the chapter on nephritis. Also since phosphorus is probably absorbed (see chapter on absorption of phosphorus) only as inorganic phosphorus, it is directly related to the phospho-proteins and phospho-lipins which must be formed from inorganic phosphorus by the body cells. So we might say that phosphorus metabolism centers around the inorganic phosphorus ion, although as will be shown later, the inorganic phosphorus may be quite normal while marked variations in the other fractions may be present. This fact is frequently not appreciated by the average practitioner.

Lohmann (36) discovered pyrophosphoric acid in muscles in 1928 and later found it to be present in the blood. Its func-

tion in the blood is not definitely known but in muscles it is thought to be part of the co-enzyme system necessary for sugar metabolism. Theoretically it could hold the same position in blood sugar metabolism, however whether phosphorus enters into the chemistry of blood glycolysis or not is still a question. The majority of investigators are in favor of the affirmative.

Emden and Zimmerman (12) in 1927 showed that resting muscles contained a hexosemonophosphoric ester. Its isolation from blood soon followed.

Jost (26) in 1927 found the greater part of the acid soluble organic phosphorus of the red blood cells was diphosphoglyceric acid. This compound is probably related to blood glycolysis and is thought to be the mother substance of urinary phosphate (see chapter on nephritis).

Hexosediphosphate (also an intermediary in sugar metabolism) may exist in the red blood cells but has never been isolated from them.

There are in all probability other phosphorus compounds present in small amounts but the sum of these compounds agrees quite closely with the total phosphorus.

Determination of Inorganic Phosphate in Blood Serum

This method is based on the Kuttner and Lichtenstein (32) modification of the Kuttner and Cohen (31) method of determining inorganic phosphate.

The Reagents;

1. Molybdic-Sulfuric Acid Mixture

(a). 10 n H₂SO₄, or the following which is nearly 10 n and will give satisfactory results. Pour 282 cc. of concentrated H₂SO₄ (95 per cent, sp. gr. 1.84) into about 600cc. of distilled water, cool, transfer to a liter flask, and make up

to volume with distilled water.

(b). 7.5 Per Cent Solution of Sodium Molybdate. -- Dissolve 7.5 gm. of Kahlbaum "Zur Analyse" or Eimer and Amend c. p. sodium molybdate in water in a 100 cc. graduated flask and make up to volume with distilled water. The products mentioned have been found reliable. Sodium molybdate obtained from other sources when used to prepare the reagent frequently gives a blue color to the blank test. This is due to soluble tungsten salts or to a substance, containing iron, tungsten, and silica, insoluble in water but soluble in sulfuric acid. If only the latter impurity is present, it will separate out and can be removed by decantation or centrifugation.

These solutions will keep indefinitely in glass-stoppered bottles. The molybdic-sulfuric acid mixture as described by Kuttner and Cohen will keep for several months. We prefer, however, to keep the sulfuric acid, 10 n, and sodium molybdate, 7.5 per cent, in separate containers as stock solutions. If only an occasional determination is made, they are added separately to the phosphate in a manner to be described below. When many determinations are necessary, the solutions may be combined for convenience as follows:

1 volume of 10 n sulfuric acid is diluted with 2 volumes of distilled water. Allow to cool, if necessary, and pour into 1 volume of 7.5 per cent sodium molybdate. Although the mixture thus prepared will keep several months, we prefer to use it freshly made. The alternative method of preparing the mixed reagent, as proposed in the original paper, by dissolving the sodium molybdate salt in 2.5 n H_2SO_4 has been rejected.

2 Stannous Chloride Stock Solution

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This is 40 per cent stannous chloride in concentrated hydrochloric acid. Dissolve 10 gm. of stannous chloride in 25 cc. of HCl. Smaller quantities may be prepared if desired. Store in brown glass-stoppered bottle and keep away from heat. We prefer to prepare the stock solution every 4 to 6 weeks although it may keep longer. For immediate use only, dilute 1 part of stock solution in 200 parts of distilled water. Any unused portions should be discarded.

3. Standard Phosphate Stock Solution

Dissolve 0.4394 gm. of dried monopotassium phosphate in 1 liter of distilled water, and add a few drops of chloroform to prevent mold formation. 1cc. = 0.1 mg. of phosphorus. Make two standard phosphate solutions by diluting 3 cc. and 6 cc. in 100 cc. graduated flasks and fill to the mark with water. The solutions contain 0.003 and 0.006 mg. of phosphorus per cc.

4. Ten Per Cent Trichloroacetic Acid

Procedure

Take 2 cc. blood serum in a 10 cc. test tube. Add 2 cc. 10 % trichloroacetic acid. Close the tube with a stopper and shake. Allow to stand a few minutes and filter. Use 1 cc. of the filtrate. The sample may contain between 0.01 and 0.05 mg. of phosphorus. (1) Transfer the 1 cc of the filtrate to be examined to a test-tube graduated at 5 and 10 cc. Add 4 cc. distilled water. (2) Place 5 cc. of each standard phosphate solution in two other similarly graduated test-tubes so that one tube contains 0.015 mg. and the other 0.03 mg. of phosphorus. (3) Add to each tube 4 cc. of the mixed molybdic-sulfuric acid reagent and mix. As an alternative procedure add to each tube 1 cc. of 10N H₂SO₄ 2 cc. of distilled water (mix contents), and 1 cc. of 7.5 per cent sodium molybdate solution. Close

with a rubber stopper and invert. (4) Then add 1 cc. of the diluted stannous chloride reagent to each tube. Immediately invert once or twice. The colors can be compared at once or within the next 2 hours in the usual manner according to the type of instrument used.

Computation

Using colorimeter with standard at 20

$$\frac{20 \times \text{amount of P in standard} \times 100}{\text{Unknown Reading} \times \text{amount of original sample used}} = \text{Mgs P per 100 cc.}$$

Absorption of Phosphorus from the Alimentary Tract

Meigs, Blatherwick and Cary (43) produced convincing evidence that phosphorus from the digestive tract reaches the general circulation only in the form of inorganic phosphate. This is confirmed by Williams (56) .

There are several factors however which influence the absorption of phosphorus. Some thought vitamin D or cod liver oil had some effect on the intestinal mucosa, favoring absorption but this has been largely discarded.

Constipation of course decreases absorption. Disordered fat digestion as in obstructive jaundice causes more of the phosphorus to be absorbed than normally.

Decreased absorption occurs when the intestinal tract becomes alkaline at too high a level precipitating the phosphorus as $\text{Ca}_3(\text{PO}_4)_2$. This condition may be eliminated by administration of acid or acid forming foods such as lactose and other carbohydrates.

Phosphatases in the intestinal mucosa hydrolyze the phosphoric esters of the food. Therefore the more difficultly hydrolyzable esters will not be hydrolyzed so rapidly or at so high a level in the intestinal tract. Milk contains, in addition to caseinogen, calcium phosphate in at least seven other forms of combination. Some are quite easily hydrolyzed. The peculiar ease with which calcium and phosphorus of milk are taken up from the intestine is probably dependent upon these facts.

Phosphorus Requirements in Man

Phosphorus is ingested in the food as nucleoprotein (meat), phosphoprotein (milk), lecithin (egg yolk, liver) and as inorganic phosphate. The following table from Kugelmass (30) shows the phosphorus content of various foods.

Phosphorus Content of Foods in Milligrams Per Cent

Cheese -----	683
Egg yolk-----	524
Beans, dried-----	471
Almonds-----	465
Wheat, whole-----	423
Peanuts-----	399
Oatmeal-----	392
Walnuts-----	357
Beef, lean-----	218
Eggs-----	180
Prunes, dried-----	105
Rice, polished-----	96
Milk-----	93
Flour, white-----	92
Potatoes-----	58
Carrots-----	46
Turnips-----	46
Beets-----	39
Bananas-----	31
Oranges-----	16
Apples-----	12

Bloom (4) has shown that cows milk may be deficient in inorganic ingredients and may contain incorrect ratio of phosphorus and calcium.

Phosphorus is probably only absorbed as inorganic phosphate (page9), the abundant intestinal phosphatases hydrolyzing all the esterified phosphorus compounds in the food.

Sherman (52) found that about .88 gms of phosphorus were the minimum requirement for maintenance. He also found that the phosphorus may all be supplied in the form of inorganic phosphorus, the body being able to synthesize all the necessary phosphorus esters.

Morse (44) also set .88 gms of phosphorus as a minimum requirement for maintenance. He found an average of .78 gms excreted in the urine. He suggest 1.5 gms as a safe daily intake.

Although the diet may contain enough phosphorus, lack of absorption may easily lead to phosphorus deficiency (see chapter on phosphorus absorption).

Weston (54) in 1931, always interested in the inorganic content of food, states "calcium and phosphorus have been studied over a longer period of time and until recently more intensively than have any of the essential mineral elements. The same striking variations are found in different samples of milk analyzed as occur with iron and iodine----- It is our observation that in those localities in which are found a high calcium and phosphorus content of milk, growth appears to be stimulated and when adult life is reached the stature is much above the average and rickets is comparatively rare.

In case of phosphorus deficiency in the diet one can easily supply phosphorus in the form of one of its inorganic calcium or sodium salts or as glycerophosphate as will be shown in the chapter on rickets.

Children especially, should be fed an abundant amount of the protective foods--milk, green vegetables, and fruits. The belief that most diets contain enough phosphorus should not be too universally accepted, because, as has been shown above, there may be an actual phosphorus deficiency.

The best index as to whether there is an actual phosphorus deficiency is by determination of the inorganic blood phosphorus because the phosphorus may be ingested in the food but still not absorbed. Therefore in many borderline cases of rickets, though on sufficient cod liver oil and high phosphorus diets, blood phosphorus determinations should be made to check the absorption of phosphorus.

Excretion of Phosphorus

Normally about .78 gms (average) of phosphorus are excreted daily in the urine, (Morse (44)). The rest is excreted in the feces as the triple calcium phosphate. However in some conditions such as in obstructive jaundice, due to lack of bile salts and interruption of fat digestion, the phosphorus is absorbed and excreted in the urine.

Excretion of phosphorus in the urine plays an important part in the acid-base equilibrium as will be shown later. In nephritis this function is seriously interfered with, (see chapter on nephritis).

Jost (26) found diphospho-glyceric acid to be easily hydrolyzed by the kidney phosphatase and brought forth the hypothesis that this phosphorus ester was the mother substance of urinary phosphates. He found that hydrolysis of the ester was stopped by an acid reaction and by the calcium ion.

Loeb (35) has defined phosphaturia as the excretion of an alkaline urine, which is turbid because of the precipitation of basic phosphates. Phosphaturia is not a pathological entity, but is a normal chemical response to the ingestion of large amounts of alkali or to a loss of acid radicals from the body.

Urinary infections such as cystitis and pyelitis are frequently accompanied by phosphaturia which results in these cases from the alkalization of the urine, the change in the reaction of the urine being due to the presence of ammonia formed by bacterial activity.

Phosphaturia is often associated with stone formation in the bladder or kidney pelvis.

The treatment of the condition is dependent on the cause. In patients with a history of urinary calculi, the reaction of the urine should be watched and kept acid.

Variations in the excretion of phosphorus in disease is considered with the diseases individually.

Blood Phosphates in Rickets

The role of inorganic blood phosphate has long been considered in bone metabolism, although the exact mechanism by which calcification occurs is unknown.

In 1923, Robison (50), while studying hexosemonophosphoric acid isolated from the products of fermentation, studied the enzymatic hydrolysis of the ester. In these studies he found that enzyme from young bone rapidly hydrolyzed the ester and concluded that there might be some relationship between hexosemonophosphate and the ossification process.

Glycerophosphate was also hydrolyzed by the bone enzyme. Kay and Robison (27) in 1924 demonstrated an acid soluble organic phosphorus compound of the blood which was hydrolyzed rapidly by the bone enzyme. The amount of ester hydrolyzed by the bone enzyme varied between 14 and 36 per cent of the total acid soluble phosphorus. This ester was later proved by Embden and Zimmerman (12) to be hexosemonophosphoric acid.

Martland and Robison (39) found bone phosphatase wherever ossification was going on. Goodwin and Robison (14) continuing the work on blood showed that the acid soluble fraction consisted of two portions, one not hydrolyzable by bone enzyme, which did not reduce Fehling's solution, and a second fraction hydrolyzable by bone enzyme, which did reduce Fehling's.

Robison and Soames (51) showed that while calcification in vitro may occur in solutions of inorganic salts, supersaturated with respect to the bone salts, calcification will take place with lower concentrations of calcium and inorganic phosphorus, if the phosphoric acid ester glycerophosphate was

also present. The presence of magnesium inhibited calcification. Kay (29) found that in experimental rickets in rats, the acid soluble ester content of unit volume of red blood cells was markedly diminished below the normal values for control animals on a normal diet, or the values for animals receiving the rachitogenic diet together with small quantities of vitamin D. The addition of therapeutic agents to the rachitic diet caused an increase toward normal in the quantity of phosphoric acid esters in the red blood cells.

Howland and his associates (22) (23) found that in all cases of active rickets either the calcium or the inorganic phosphorus of the blood serum, or both, were diminished. In uncomplicated rickets only the inorganic phosphorus is reduced instead of the normal concentration of 4 to 6 mgs % this may be as low as 2 mgs % or even lower. The calcium is unaffected except in cases of tetany. He also points out the unstable condition of these minerals in blood serum in active rickets, where an increase in the phosphorus intake may raise the blood phosphorus but at the same time cause a lowering of the blood calcium enough (below 7) to bring on tetany. The parathyroid is known to have a stabilizing effect on the level of blood calcium and phosphorus. Excessive doses of parathormone may cause decalcification of the bones and pathological calcification in the soft tissues.

Greenwald and Gross (15) showed that in long continued administration of parathyroid extract there was an increased excretion of both calcium and phosphorus, which can continue for a long time. He had shown previously (17) (18) a marked

increase in the acid soluble fraction of blood phosphates after parathyroidectomy. But in the gastrocnemii of parathyroidectomized dogs, Dixon and Ranson (10) (11) found no changes in the acid soluble content nor in the partition of phosphorus compounds.

Bakwin, Bodansky and Turner (3) found that the average of inorganic blood phosphorus and pyrophosphate in whole blood in normal infants was 3.9 and 4.8 Mgs % respectively, while the average in rachitic infants for the inorganic phosphorus and pyrophosphate was 2.6 and 3.8 Mgs % respectively.

Bloom (4) showed that milk used for human consumption exhibited deficiency of inorganic ingredients and incorrect ratios, and showed that rickets in children has been prevented and cured by administration of dicalcium phosphate and concluded that dicalcium phosphate, when added to the diet of infants and children, would complete the rachitic prophylactic trinity--inorganic salt, vitamins, sunshine.

Lecoq and Villuis (34) showed that the sodium and calcium glycerophosphates had an antirachitic action equal to calcium monophosphate for the same amount of phosphate present and seemed to have about twice as great an effect as dicalcium phosphate.

From the above articles, the author concludes, that in the prevention and treatment of rickets, phosphate determinations are of great value. When the blood phosphate value is below normal some form of phosphate may be added to the diet, more cod liver oil given and the absorption of phosphate from the alimentary canal should be checked as previously described in the chapter on absorption of phosphorus.

The exact role played by the acid soluble organic phosphate esters is not entirely understood but it is probably by the hydrolysis of them by the bone phosphatase, producing a supersaturation of inorganic phosphorus, that bone deposition occurs.

It must also be realized that vitamin D will not cure all cases of rickets, why we do not know, but in these, both inorganic phosphorus and calcium determinations should certainly be made.

The parathyroid effect should also be considered.

Osteitis Fibrosa Diffusa and Hyperparathyroidism

Functional over activity of the parathyroid is now a well recognized condition. Under the influence of the parathyroid hormone the calcium of the bones is mobilized and excreted in increased quantity, the bones become decalcified (osteitis fibrosa diffusa), and the blood calcium rises. The blood phosphate is low and may fall to 1.0 mg per cent, Byrom (9). In some cases an adenomatous tumor of the parathyroid is present, and if this is excised, the blood phosphate rapidly returns to normal.

Therefore in all cases of osteitis fibrosa diffusa or suspected cases, blood phosphorus determinations should be made as a tumor of the parathyroid might be diagnosed this way.

Tetany

Tetany (2) is a symptom-complex occurring under widely varying circumstances. It consists of a great hyperexcitability of the neuromuscular apparatus which manifests itself by intermitting spasms of the muscles.

These spasms may affect any muscles of the body, but they most commonly occur in the periphery of the limbs. They are usually bilateral, in severe cases painful, and they may last for ten to twenty minutes and recur over many weeks.

An attack begins with a sensation of tingling and stiffness in the fingers. The thumb is forcibly adducted, the fingers pressed closely together, being flexed at the metacarpophalangeal joints and extended at the interphalangeal joints. In severe cases the wrists and elbow may be flexed and the shoulders adducted. When the lower limb is affected the toes and ankles are plantar-flexed, the soles of the feet hollowed out, and the knees and hips extended.

Laryngospasm may occur in tetany. In rickets, it may occur without the occurrence of tetany. In tetany the muscles of the trunk are involved only rarely. The muscles of the face are sometimes involved, and spasm of the oculomotor muscles may occur sufficient to cause diplopia.

Tetany occurs in widely different diseases as rickets and pyloric obstruction, but chemical studies have enabled us to divide cases of tetany into 2 groups, namely, those with a low serum calcium and those with an alkalemia.

The reciprocal relations of blood phosphorus and calcium have long been known. Conditions, producing a phosphate retention or increase, produce a low serum calcium.

The following conditions are characterized by a low serum calcium and high serum phosphate.

1. Experimental parathyroidectomy
2. Following operations upon thyroid gland with parathyroid damage.
3. Parathyroid tumor
4. Spontaneous hypoparathyroidism
5. Rickets--a small proportion have low serum calciums but this is not the rule. (See chapter on rickets.)
6. Osteomalacia
7. Hunger osteopathy
8. Lactation--may accelerate the course of osteomalacia
9. Idiopathic steatorrheas--celiac disease of children, non-tropical sprue and sprue appear to be due to defective absorption of fat. This defect interferes with the absorption of Vitamin D. so for this reason the serum calcium is low.
10. Chronic nephritis. (see chapter on nephritis)

In diagnosis, treatment and prognosis of the above diseases, blood calcium, and inorganic phosphorus determinations are of value.

Blood Phosphates in Diabetes

A relationship between carbohydrate metabolism and phosphorus has been recognized for over 30 years. Harden and Young (19) found a disappearance of inorganic phosphate during yeast fermentation in 1908 and isolated and indentified hexosediphosphoric acid two years later. Hesosephosphate compounds were later found in muscle.

Fiske (13) was perhaps the first to call attention to the decreased output of urinary phosphates after sugar ingestion.

Wigglerworth, Woodrow, Smith and Winter (55), Harrop and Benedict (20) were among the first to demonstrate a relationship between blood P and carbohydrate metabolism. They found a definite fall in the concentration of blood P following insulin injections.

Naturally phosphorus is important in the chemical studies of diabetes.

Allan(1) found a continuous high urinary excretion of phosphorus in phlorhizin diabetes and an actual phosphorus deficiency in diabetes mellitus.

Bolliger and Hartman (7) in 1925 studied blood sugar and blood phosphorus curves simultaneously after glucose, insulin and adrenalin injections on partially and completely depancreatized dogs and on human subjects. They arrived at the following conclusions.

1. Blood phosphates are depressed during carbohydrate metabolism only when insulin is available.
2. The complete absence of pancreatic hormone is shown by the blood phosphate level which remains unaffected as a straight line during carbohydrate metabolism.
3. As concerns phosphate metabolism, pituitrin is a

direct antagonist of insulin.

4. Carbohydrates and phosphates are best utilized when there is an excess of each in the circulation.

They maintained that the variation in the blood phosphate curve is often more apparent than the variation from normal in the corresponding blood sugar curve in mild borderline cases.

Mark and Morgan (37) injected hexosediphosphoric and hexosemonophosphoric acid in animals with insulin hypoglycemia, but obtained no effect while glucose injections would immediately relieve the condition.

Meier and Thoenes (42) fractionated the acid soluble phosphorus compounds in diabetics with the following outstanding results.

	Blood Sugar in Mgs %	Lactic Acid in Mgs %	Mgs % $P_2O_5^*$	Inorg anic	Hydrolysis 7 min	after 3 hours	Total Acid Soluble
Normal-----	103.5	12.8	8.5	12.3	13.0	21.7	54.5
Mild Diabetes-----	182.0	14.9	6.6	12.3	11.3	21.4	51.5
Severe Diabetes-----	229.0	19.4	6.3	12.0	8.7	16.0	43.0
Diabetic Coma-----	526.0	40.2	9.8	11.7	6.2	8.3	35.8
Coma Treated by Insulin-----	300.0	15.8	5.3	9.7	9.2	16.3	40.2
Coma Well-treated by "-----	186.5	13.7	8.8	10.5	11.5	20.0	51.2
Diabetes Treated by "-----	155.0	12.4	8.3	12.0	12.6	19.9	52.8

The table shows the marked increase in lactic acid and slight increase in inorganic P in diabetic coma with a marked reduction in the ester difficult to hydrolyse and in the total acid soluble P. Insulin restored the various fractions to

*The Germans express P in Mgs % P_2O_5 instead PO_4

their normal limits. From these results, it is seen that insulin influences not only the inorganic P, but the other fractions of the acid soluble P as well. These results would also tend to show that the hexose phosphates of the blood play a part in carbohydrate metabolism, particularly the ester difficult to hydrolyse which I consider to be diphosphoglyceric acid and hexosemonophosphoric acid, although there may be some other as yet unidentified compounds also.

Sherman Pinto (49) reviewed the literature on glycolysis in diabetic blood in vitro and found about half the investigators found glycolysis decreased and half found it normal. The investigations seemed to point to the hypothesis that the ability of the blood to glycolyze depended inversely on the concentration of ketone bodies present, however the question is not definitely settled.

McCullagh and Van Alstine (40) studied the relationship between phosphate and blood sugar curves in sugar tolerance tests. They did not find a definite variation in the curves of diabetic patients as Hartman and Bolliger (7) had previously reported, however they gave their glucose by mouth while Hartman and Bolliger gave the glucose intravenously. Hartman and Foster (21) in another communication presented a study of 500 clinical cases of incipient or prediabetic patients, studying the blood phosphate and sugar curves, following intravenous injections of glucose. They found as Hartman and Bolliger had previously reported that the curve of inorganic phosphates was a valuable supplement to the glucose tolerance curve in the diagnosis of abnormal carbohydrate

metabolism.

Byrom (9) has stated that in diabetes no significant alteration occurs in the blood phosphorus unless there is severe ketosis or coma. When these complications are present there is a marked fall in the ester phosphorus of the corpuscles, similar to that observed in ammonium chloride acidosis. Effective insulin treatment restores the normal phosphorus balance. In neglected diabetic coma the inorganic phosphate in the blood may rise to 8 mg. per cent or more, renal damage probably being responsible, since the blood urea is usually raised in such cases. A high blood phosphate in a case of diabetic coma implies that recovery is extremely unlikely.

Joslin (25) with the aid of Bloor and Gray demonstrated an increase in blood fat in diabetes and a corresponding increase in the lecithin value. These values varied greatly with diet and treatment.

Further studies of the blood phosphorus compounds, especially the acid soluble esters, should be made as Meier and Thoenes (42) have shown them to vary markedly in diabetes.

Blood Phosphates in Nephritis

Greenwald (18) while studying blood phosphorus compounds noted an increase of total phosphorus in the blood serum in some cases of nephritis and with this there was often an increase in his so-called acid soluble fraction, which he believed represented chiefly inorganic phosphates. This was in 1915.

Marriott and Howland (38) in 1916 found an increase in the inorganic phosphorus in the serum of patients with acidosis occurring in nephritis. They found as much as 23 Mgs per cent inorganic phosphorus in the serum of some of the more severe cases. They attributed this retention as being due to a certain specificity of retention because in some cases of retention, there was no increase in sodium chloride. There was also no relation to total nitrogen and urea retention. In the acidosis of diabetes they did not find a phosphate retention, (see chapter on diabetes). The urinary output in the cases of phosphorus retention was not increased and in some cases was diminished so they concluded that the phosphate retention was due to an interference with a specific function of the kidney. Associated with the phosphate retention, they found a marked reduction of calcium. Administration of calcium produced an increased elimination of phosphate.

Kay (28) produced acidosis by NH_4Cl and found a marked decrease in the stable ester of the acid soluble fraction (diphosphoglyceric acid) of the blood cells and an increase in urinary excretion following this drop.

Jost (26) found the greater part of the acid soluble organic phosphorus to be diphosphoglyceric acid, which is eas-

ily hydrolyzed by the kidney phosphatase. He believed diphosphoglyceric acid therefore to be the mother substance of urinary phosphates and consequently of great importance in the acid-base equilibrium of the blood. This ester has also been shown to have some relation to carbohydrate metabolism.

Osman (46,47,48,) has shown the alkali treatment in Bright's Disease in combating the low plasma bicarbonate due to phosphorus retention and other anions to be applicable in cases of chronic parenchymatous nephritis, chronic "mixed" nephritis, the late stages of acute parenchymatous nephritis and in the later stages of sub-acute nephritis with persistent edema and without marked hematuria. A preliminary estimation of the plasma bicarbonate should be made.

As previously discussed, the phosphate ion plays an important part in the acid base balance. It is an important buffer, having a value of about 20% of the total buffering power of the blood. Its action as a buffer is as follows $B_2HPO_4 + HA = BH_2PO_4 + BA$, example $(Na_2HPO_4 + H_2CO_3 = NaH_2PO_4 + NaHCO_3)$. The acid phosphate is then passed into the urine and lost from the body. Thus we see a daily absorption and excretion is part of the normal mechanism in the acid base regulation. In kidney damage, especially the parenchymatous type of nephritis or the acidotic type, the ability of the kidney to excrete phosphate is impaired. Therefore in the treatment of such types of nephritis, phosphate determinations often give a better index to the severity of the disease than urea and non-protein nitrogen determinations, the latter often not being increased in this type of nephritis. Therefore phosphate determinations are of value in the diagnosis, treatment and prognosis in this type of nephritis. Too often only the

urea and N.P.N. values are considered to be of importance in judging the condition of the kidney.

Byrom (9) has stated "Extensive destruction of renal tissue, from whatever cause, is accompanied by a diminished excretion of phosphate in the urine and a rise in the blood phosphate. The degree of phosphate retention is roughly proportional to the severity of the disease, as judged clinically and by the urea-concentration test. In uremia, readings of 20-30 mgs. per cent are not uncommon, and a blood phosphate of 8 mgs. per cent or over usually foreshadows death within a few weeks. In hydraemic forms of nephritis (and rarely in uraemia) the phosphate is normal. This retention of acid phosphates is responsible for the acidosis and hyperpnoea (renal asthma) which are common in uremic nephritis, and also causes secondary changes in calcium metabolism. The blood calcium falls considerably and in children ossification may be defective (renal rickets). Large doses of alkali sometimes relieve the symptoms of phosphate retention, but are apt to precipitate tetany. A more rational method of treatment is to hinder the absorption of phosphate from the intestine and this can be accomplished by oral administration of calcium lactate."

Chronic Myelogenous Leukemia

Buckman, Doland and Weed (8) found both total and inorganic phosphorus of the cells greatly increased in chronic myelogenous leukemia. Following irradiation, there was a marked drop to almost normal limits as the white cells and immature cells diminished.

Nucleated cells and immature cells respire more than non-nucleated cells or old cells. This would explain a greater need for phosphorus for the increased aerobic glycolysis and respiration.

The increase of nucleated cells would of course account for most of the increased phosphorus.

Labbe, Petresco and Fabrykant (33) concluded that the phosphorus variations, exceptin the inorganic, were due to quantitative and qualitative changes in the red and white cells.

Blood Phosphates in Anemia

Bloor (5) produced acute experimental anemia in rabbits and found the lipid phosphorus of the plasma was increased 6 to 7 times the normal, while the lipid phosphorus of the cells was increased 2 or 3 times. The inorganic phosphorus was increased some, while the total acid soluble organic phosphorus remained about the same. He also contended that the lipid phosphorus was higher in newly formed cells than in old ones.

Labbe, Petresco, and Fabrykant (33) concluded that all the variations of blood phosphorus and its fractions, except the inorganic, in the leukemias and anemias were due to quantitative and qualitative changes of the red and white cells.

Muller and Heath (45) studied plasma cholesterol and lecithin in various blood conditions and concluded that the plasma lipoids were not related to anemia per se, but definite characteristics were obtained in certain blood disorders. In anemia due to acute loss of blood, the lipoids remained at normal levels or were high, while in that caused by chronic loss of blood, they tended to be low at the height of the anemia, but increased with improvement after the reticulocyte response had subsided. This is in contrast to their behavior in pernicious anemia, in which the increase of the lipoids parallels the reticulocyte response.

In carcinoma of the stomach associated with anemia, the lipoids remained low except when the anemia decreased.

In chronic myelogenous leukemia, a dissociation be-

tween plasma cholesterol and lecithin phosphorus occurred. The level of cholesterol was found to be subnormal, or at the lower limit of normal, even when no anemia was present, while the lecithin phosphorus was normal. In aplastic anemia the lipoids were high in spite of severe anemia.

Phosphorus Poisoning

This condition formerly was quite common in the United States, until the use of white phosphorus was prohibited or guarded against. Characteristic changes occur in the liver in acute cases, while bone changes are common in chronic cases. While the condition is rare now, accidental cases occasionally occur.

In phosphorus poisoning, toxicologists have never been able to find much change in the inorganic phosphorus content of the blood or any of the metallic phosphorus, the latter being oxidized rapidly and excreted.

McClellan, MacDonald, and Sullivan (41) studied a case of phosphorus poisoning in an 18 months child from the ingestion of roach paste. The child died and autopsy was done. Before the child died various chemical studies of the blood were made. The plasma inorganic phosphorus was considerably reduced (2.7 mgs per 100 cc) normal being 5 to 6 mgs %. The child clinically and by roentgenograms showed no signs of rickets. They attributed this lowering to the failure of carbohydrate metabolism. The blood sugar was 55 Mgs %.

I was unable to find in the literature any work on the other phosphorus compounds of the blood in phosphorus poisoning besides the inorganic phosphorus.

Conclusions

1. Inorganic phosphorus determinations of the blood are of great value in rickets, diabetes, hyperparathyroidism, nephritis, and tetany.
2. Inorganic phosphorus determinations are of practically no value in cases of phosphorus poisoning.
3. Organic phosphorus determinations may prove to be of clinical value in rickets and diabetes.
4. Phosphaturia is a symptom and not a disease.
5. Cow's milk may be deficient in phosphorus
6. Faulty absorption of phosphorus from the intestinal tract may produce rickets.
7. Restriction of phosphate absorption from the intestinal tract is of value in the acidotic type of nephritis.
8. Phosphorus may be supplied in the form of sodium or calcium diphosphate or as glycerophosphate in phosphorus deficiency.
9. Inorganic phosphorus determinations of the blood are easy to make and should be made more frequently than they are.

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